Experimental studies on the combined chemotherapy with anticoagulant and anticancer agent

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Experimental studies on the combined chemotherapy with anticoagulant and anticancer agent

by

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I. INTRODUCTION

As early as 1869, T. R. ASHWORTH[1] demonstrated the tumor cells in the circulating blood in the patients with malignant tumors. Since then, it has been examined in greater detail and confirmed by many investigators experimentally[2] as well as clinically[3].

However, ENGELL[4] postulated that tumor cells in the circulating blood did not always lead to metastasis and evidently parts of them were destroyed in blood vessels. In general, pathological processes of the metastasis formation has shown that tumor cells injected experimentally into blood vessels or drained in the veins are adhered at first to the capillary walls, where they become entrapped in fibrin or small thrombi and then are fixed[5]. Therefore, combined chemotherapy with anticoagulant and anticancer agent for malignant tumors seem to present interesting problems: that is, when anticoagulants are used for the prevention of metastasis or for therapy of relatively fresh small metastatic lesions, anticancer agents may act more effectively against tumor growth.

Considering the pathological processes of metastasis formation, the author in his experiments attempts to evaluate the effect of combined therapy with chemotherapeutic agent and anticoagulant on the production of hematogenous metastases.

II. MATERIALS AND EXPERIMENTAL CONDITIONS

1. Materials

1) Animals and tumor: The animals used in the present study were rats weighing about 100 g of Donryu strain. The tumor used was Yoshida sarcoma in the ascitic form. For these experiments the fluid was diluted with saline to a count of 300,000 cells/cc and 1 cc of the tumor cell suspension was injected.

2) Anticancer agent: Mitomycin C was used and 2 mg of it was diluted with 5.0 cc of saline and its dosage was adjusted according to the following conditions.

3) Anticoagulant: 40 units of sodium heparin was injected intravenously, once for each animal.

4) Proteolytic enzyme: 5 units chymotrypsin was injected subcutaneously for every 1 kg of body weight.

2. Experimental conditions

In order to produce the hematogenous metastases, 300,000 cells of Yoshida sarcoma were injected intravenously into the right femoral vein. The animals were divided into four groups. Animals in all groups were observed for 30 days and then were sacrificed and autopsied. The incidence of metastases and survival period and others were observed.

Group I: Mitomycin C was injected intraperitoneally at the same time with intravenous injection of tumor cells. The administered dosis of Mitomycin C were divided into following 3 groups; 0.5 mg/kg, 1.0 mg/kg and 2.0 mg/kg.

Group II: Both heparin and tumor cells were injected intravenously at the same time.

Group III: Both heparin and tumor cells were injected intravenously at the same time with intraperitoneal injection of 1.0 mg/kg of Mitomycin C.

Group IV: Chymotrypsin was injected subcutaneously at the same time with intravenous injection of tumor cells.

In the experiments the seasons and breeding circumstances were taken into consideration, in which the experiments were carried out, and the control was made in each group and the tumor cells were inoculated only intravenously.

III. RESULTS

Group I: Results of the intravenous injection of tumor cells and simultaneous intraperitoneal injection of mitomycin C are shown in Table 1.

In the group, in which 0.5 mg/kg of mitomycin C was given, one out of the 20 animals survived over 30 days. Except the one that survived long, the survival periods were from 7 to 13 days, and the average period of survival was 10.6 days. The periods of survival in the control group were from 6 to 14 days, and the average was 9.6 days.

In the group, in which 1.0 mg/kg of mitomycin C was given, the long survivors numbered 2, and except for these cases, the survival period was from 7 to 17 days, the average being 9.5 days, and only 2 out of 25 animals (8%) showed a long period of survival. In the control group the survival period was from 5 to 15 days, the average being 6.9 days.

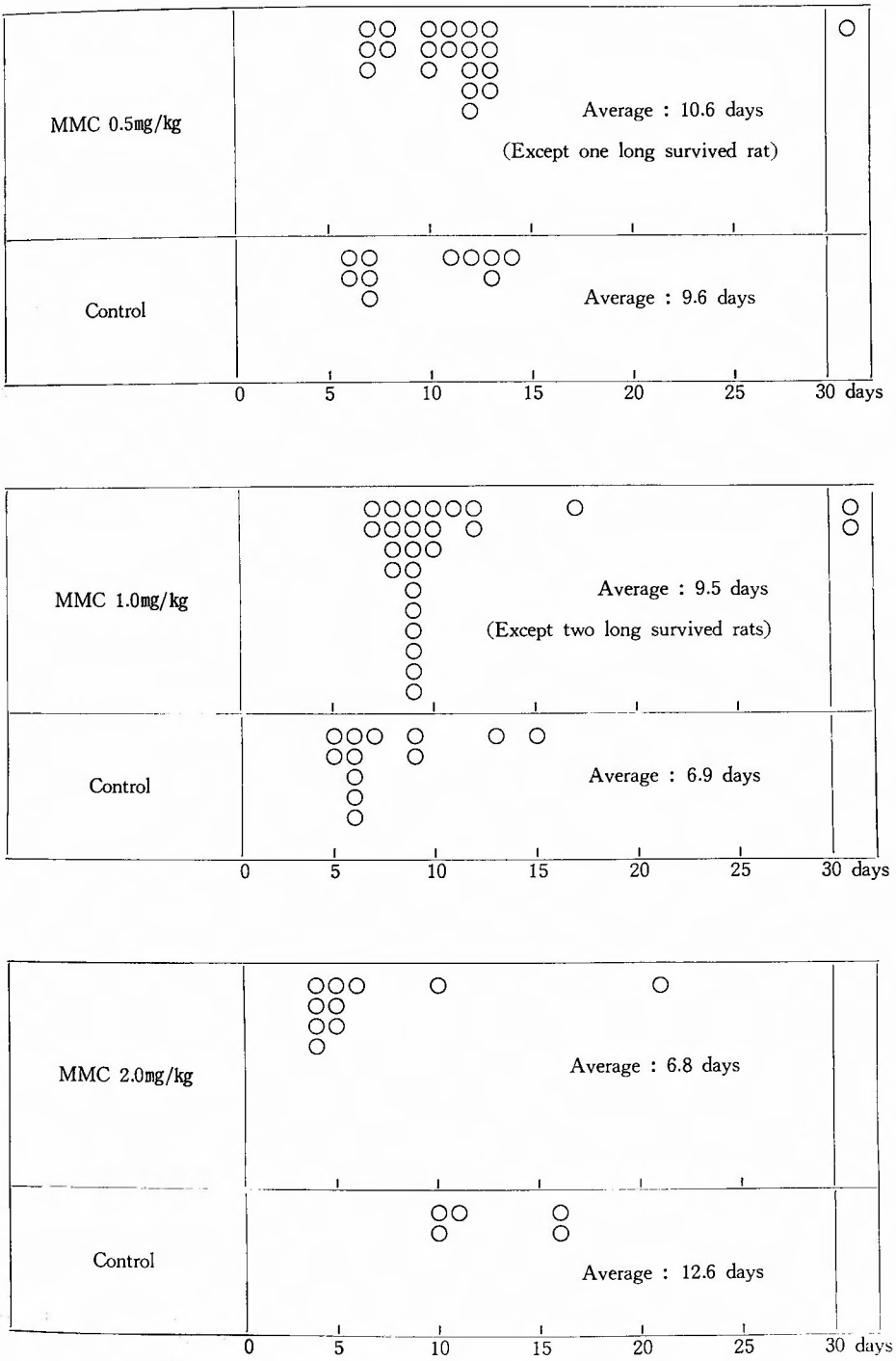
In the group, to which 2.0 mg/kg of mitomycin C was given, the survival period was from 4 to 21 days, and the average was 6.8 days, and there were no long survivors. In the control group the survival days reached from 10 to 16 days, and the average showed 12.6 days.

One long survival in the group injected with 0.5 mg/kg of mitomycin C and two long survivals in the group injected with 1.0 mg/kg were sacrificed on the 30th day and examined microscopically on the presence of metastasis. The results are shown in Table 2 and 3. One out of 3 cases had obvious metastasis, but the other two cases had no tumor cells microscopically in the various organs.

Summarizing the results of these groups, it seems that it is most effective to use 1.0 mg/kg of mitomycin C judging from the average survival days and also from the survival rate in the case of long survivors.

Group II: The survival days in the group injected simultaneously with tumor cells and heparin intravenously are shown in Table 4. It is clear that the survival days reached from 6 to 30 days, except 3 animals of long survival, and the average was 13.2 days.

Table 1 Survival days in the groups treated with mitomycin C



MMC : Mitomycin C

Table 2 Existence of metastasis in the group treated with mitomycin C 0.5mg/kg

Organ \ Existence of metastasis \ Treated group			Survived over 30 days	Died within 30 days	Control
Lung	Macroscopic metastasis		−	−～+	−～+
	Microscopic metastasis	Perivascular or peribronchial region	−	++～+++	+++
		Intravascular or intracapillary space	−	+～+++	−～+++
		Intratracheal space	−	−～+	−～+
Liver	Macroscopic metastasis		−	−～+	−～+
	Microscopic metastasis	Glisson's area	−	+++	+++
		Parenchymal sinusoid	−	+～+++	++～+++
Kidney	Macroscopic metastasis		−	−～+	−～+
	Microscopic metastasis	Intracapillary in the glomerular area	−	−～+	−～+
		Peritubular region	−	−～+++	−～+++

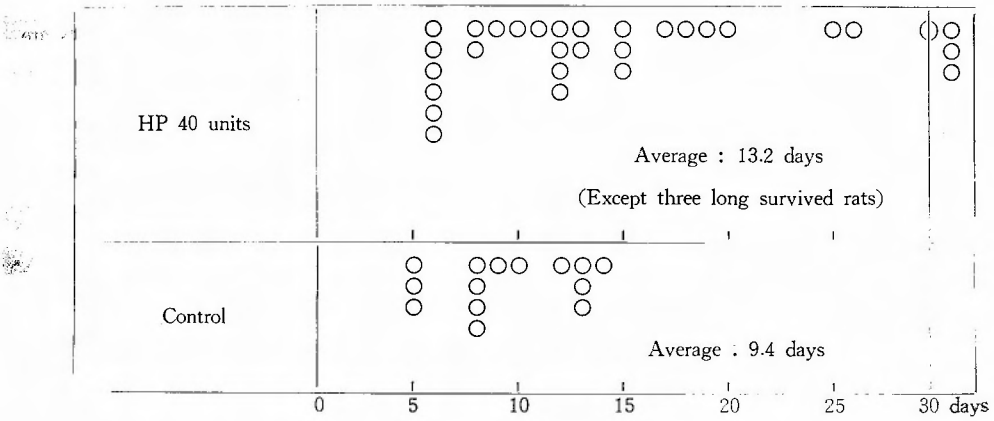
Macroscopic metastasis :
+ . Presence of metastatic nodules in the cut surface of organs.
− : Absence of metastatic nodules in the cut surface of organs.
Microscopic metastasis :
+++ : Infiltration of numerous tumor cells. ++ : Infiltration of several tumor cells.
+ . One or two tumor cells. − : Absence of tumor cells.

Table 3 Existence of metastasis in the group treated with mitomycin C 1.0mg/kg

Organ \ Existence of metastasis \ Treated group			Survived over 30 days		Died within 30 days	Control
Lung	Macroscopic metastasis		−	+	−～+	−～+
	Microscopic metastasis	Perivascular or peribronchial region	−	+++	++～+++	++～+++
		Intravascular or intracapillary space	−	−	+～+++	+～+++
		Intratracheal space	−	−	−～+	−～++
Liver	Macroscopic metastasis		−	−	−～+	−～+
	Microscopic metastasis	Glisson's area	−	−	++～+++	++～+++
		Parenchymal sinusoid	−	−	+～+++	++～+++
Kidney	Macroscopic metastasis		−	−	−～+	−～+
	Microscopic metastasis	Intracapillary in the glomerular area	−	−	−～+	−～+
		Peritubular region	−	−	−～+++	−～+++

3 out of 30 animals survived over 30 days and long survivors came up to 10% of the all. The survival days in the control group showed from 5 to 14 days, and the average was 9.4 days. In the group receiving heparin only, the prolongation of survival time over the control group is evident. Three animals which survived long were sacrificed on the 30th day and examined for the existence of metastasis microscopically. The results are as follows: One animal had an accumulation of tumor cells in Glisson's area of liver but the other two had no tumor cells microscopically in the various organs (Table 5).

Table 4 Survival days in the group treated with heparin



HP : Heparin

Table 5 Existence of metastasis in the group treated with heparin

Organ	Existence of metastasis \ Treated group		Survived over 30 days			Died within 30 days	Control
Lung	Macroscopic metastasis		−	−	−	−～+	−～+
	Microscopic metastasis	Perivascular or peribronchial region	−	−	−	++～+++	+++
		Intravascular or intracapillary space	−	−	−	+～+++	++～+++
		Intratracheal space	−	−	−	−～+	−～+++
Liver	Macroscopic metastasis		−	−	−	−～+	−～+
	Microscopic metastasis	Glisson's area	−	+++	−	+～+++	+++
		Parenchymal sinusoid	−	−	−	+～++	++～+++
Kidney	Macroscopic metastasis		−	−	−	−～+	−～+
	Microscopic metastasis	Intracapillary in the glomerular area	−	−	−	−～+	−～+
		Peritubular region	−	−	−	+～+++	+～+++

Group III: From the results of Group I it became evident that administration of mitomycin C was effective in preventing the development of metastasis. Such results of experiments were identical with the reports of many researchers. It seemed to be necessary to take into consideration the dosis of mitomycin C. From the results of Group II it

became evident also that the administration of the anticoagulant was more effective to prevent the development of metastasis than that of mitomycin C alone. After considering the results of Group I and II, heparin and an adequate dosis of mitomycin C were administered in Group III. The results are shown in Table 6. Namely, in the case of the group treated with both heparin and 1.0 mg/kg of mitomycin C the survival days were from 6 to 30 days, except 8 animals which survived long, and the average was 13.5 days. A marked prolongation of survival period was seen in 8 out of the 30 animals (26.7%) of Group III and this result was remarkably better than in Group I or II. In the cases of the long survived 8 animals the histological specimens were made after sacrifice, and existence of tumor cells in various organs was examined microscopically. The results are shown in Table 7. Only in one out of 8 cases the accumulation of tumor cells was ascertained microscopically. The survival days in the control group showed from 5 to 18 days in an average of 8.7 days.

Table 6 Survival days in the group treated with heparin and mitomycin C

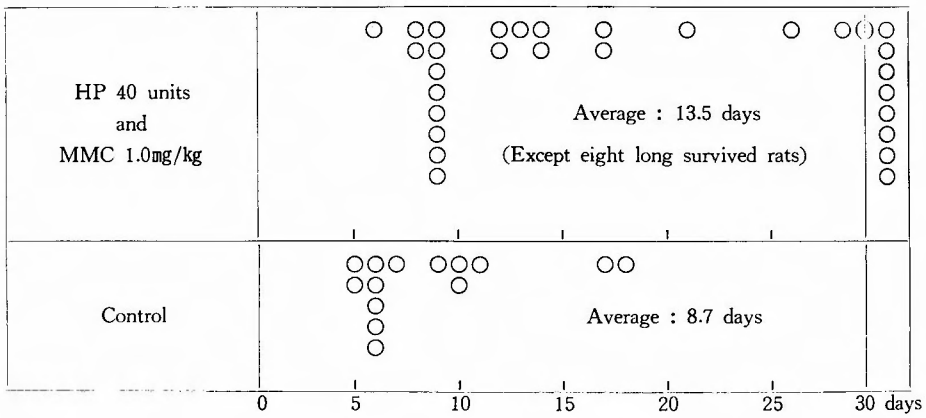
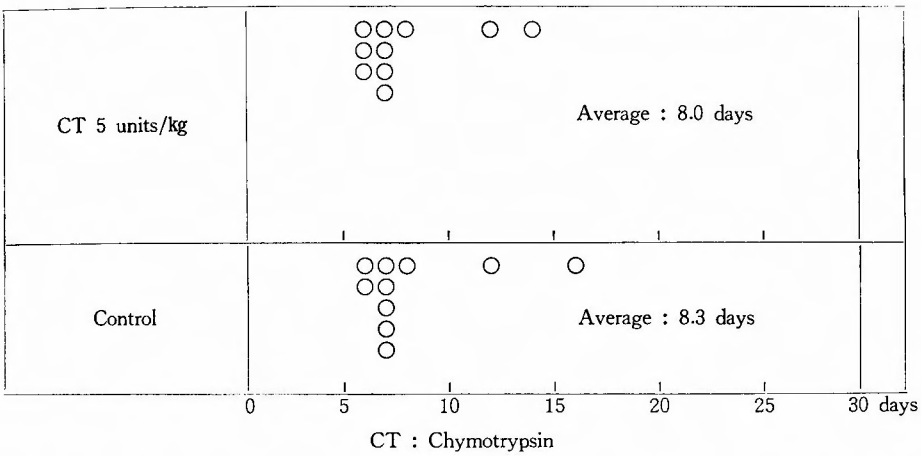


Table 7 Existence of metastasis in the group treated with heparin and mitomycin C 1.0mg/kg

Organ	Existence of metastasis \ Treated group		Survived over 30 days								Died within 30 days	Control
Lung	Macroscopic metastasis		−	−	−	−	−	−	+	−	−～+	−～+
	Microscopic metastasis	Perivascular or peribronchial region	−	−	−	−	−	−	##	−	+～##	#
		Intravascular or intracapillary space	−	−	−	−	−	−	−	−	+～#	#
		Intratracheal space	−	−	−	−	−	−	−	−	−～#	−～+
Liver	Macroscopic metastasis		−	−	−	−	−	−	+	−	−～+	−～+
	Microscopic metastasis	Glisson's area	−	−	−	−	−	−	##	−	+～##	#
		Parenchymal sinusoid	−	−	−	−	−	−	−	−	+～##	#
Kidney	Macroscopic metastasis		−	−	−	−	−	−	−	−	−～+	−～+
	Microscopic metastasis	Intracapillary in the glomerular area	−	−	−	−	−	−	−	−	−～+	−～+
		Peritubular region	−	−	−	−	−	−	−	−	+～##	−～##

Group IV: In the group treated with chymotrypsin the survival days were from 6 to 14 days. These were not different from the control group and there was no animal which survived long (Table 8).

Table 8 Survival days in the group treated with chymotrypsin



CT : Chymotrypsin

Side effects found in the group administered with heparin: Petechiae or other hemorrhagic tendency owing to the use of heparin was not seen in liveing animals and also in the autopsy findings.

IV. DISCUSSION

Literatures on the antitumor effects of mitomycin C are too many enumerated and effects for malignant tumors are generally recognized[6-10]. Generally speaking, antitumor agents are more effective when tumor cells are in the stage of free condition, and the fact is proved experimentally that its effects are less marked when tumor formation is found[11)12)].

On the other hand, it was WOOD[5)] who revealed the pathologic-anatomical process of lodgement of tumor cells. According to WOOD lodgement of tumor cells may cause the formation of fibrin or small thrombi in the capillaries. Individual tumor cells can pass through capillaries without fixation, even though they are much larger than the lumen of the capillary itself[13)]. There are some reports in which the following facts were reported: Tumor cells remain free in the circulating blood for a long period when heparin or fibrinolysin is administered and also the fibrin formation has been prevented[14)15)]. Therefore, the use of anticoagulant appears to prevent the formation of metastasis. In fact it was reported by LAWRENCE et al.[16)] in 1952 that the use of anticoagulant prevented the experimental formation of hematogenous metastasis of tumors in lung and similar reports on it were seen afterwards[17-22)].

More recently the range of experimental conditions has been expanded and the following reports are available. The use of anticoagulant was effective to prevent the increase of metastasis after the surgical stress[23)24)]. The long-term anticoagulant treatment had shown effective results in preventing metastasis[25)26)].

The above-mentioned studies showed that anticoagulant had an inhibiting effect on the development of metastasis in the experimental tumors. On the other hand, O'MEARA and O'HALLORAN[27] applied this principle clinically and reported that 6 out of 9 patients with malignancies showed regression of primary tumor and 5 patients showed regression of metastatic lymph nodes. It is well-known that in the recent years anticoagulant has been used widely for thromboembolic disease. MICHAELS[28] reported the interesting results, in which the patients treated with the anticoagulant had no difference in the incidence of cancer as compared with the control group but the incidence of metastases was evidently lower in the group of anticoagulant therapy.

Considering from the view points of the pathological and biochemical background on the development of metastasis and also from the experimental and clinical facts, the use of anticancer agent with fibrin inhibitor shall present very interesting problems on the chemotherapy for cancer. And detailed fundamental studies seem to be necessary to develop the clinical applications. However, there have been no systematic studies and very little information is available in this respect[29][30].

In this experiment anticoagulant (heparin) and anticancer agent (mitomycin C) were used together to prevent the experimental formation of metastasis and the results were stated as above. Namely, the ratio of long survivors in rats receiving injection of heparin only was 10%; for animals receiving injection of mitomycin C (1.0 mg/kg) only it was 8%; for animals receiving injection of both heparin and mitomycin C it was 26.7%. It was confirmed that not only the ratio of long survivors in the combined therapy group was much better than that in the single use of heparin or mitomycin C, but also in the average survival day it was longer than the group of administration of single agent. The use of anticoagulant and anticancer agent showed good results in preventing metastasis and also to be very effective for curing small metastasis. According to the results in which chymotrypsin, which has proteolytic but no fibrin inhibiting effect, was used, observed effects could not be recognized to prevent metastasis formation. Therefore, the reason that heparin is effective as anticancer agent, is due to the fact that it inhibits fibrin formation and further is effective for the prevention of small metastasis formattion.

The present study suggests that a new combined therapy, anticoagulant and anticancer agent, is available for preventing the lodging of metastasis or for attacking fresh small metastatic lesions.

V. CONCLUSION

The author had injected Yoshida sarcoma cells into femoral vein of rats of the Donryu strain and treated with anticoagulant and anticancer agent to prevent metastasis and to cure small metastasis, and obtained the following conclusions:

1) In the groups, in which only mitomycin C was given, the most effective dose was 1.0 mg/kg and its results showed the best survival rate and 8% of the animals showed long survival.

2) In the group, in which only heparin was given, 10% of the animals showed long survival.

3) In the group, in which both the adequate dosis of mitomycin C and heparin were given simultaneously, 26.7% of the animals showed long survival.

4) The long survived animals were examined on the metastasis in the various organs microscopically. About 90% of the animals in the group receiving both heparin and mitomycin C had no metastasis. About 60% of the animals in the group receiving heparin or mitomycin C alone had no metastasis.

5) Hemorrhagic tendencies could not be recognized in any animal under heparin treatment.

6) In the group receiving chymotrypsin the author could not obtain the same results as in the group receiving heparin. The anticancer effect of heparin seems to have a relation to interference of the fibrin formation, which is regarded as the first step of the lodgment of tumor cells at .organs.

The author showed in the present experiments that the combined therapy with anticoagulant and anticancer agent was very effective in preventing the metastasis formation of malignant tumor or for therapy of fresh small metastatic lesions.

At the end of the thesis, the author wishes to express his deepest appreciation to Professor Dr. CHUJI KIMURA for his guidance and for his kind advice on this thesis, and the author is indebted to Lecturer Dr. Ryo Inouye for the helpful encouragement and valuable advice.

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(* Written in Japanese)

Pathological findings in various organs in the cases of the long survivors treated with 1.0 mg/kg of mitomycin C.
Metastases were not found. **Fig. 1** Lung **Fig. 2** Liver **Fig. 3** Kidney
Metastases were found. **Fig. 4** Lung

Pathological findings in various organs in the cases of the long survivors treated with heparin.
Metastases were not found. **Fig. 5** Lung **Fig. 6** Liver **Fig. 7** Kidney
Metastases were found. **Fig. 8** Liver

Pathological findings in various organs in the cases of the long survivors treated with heparin together with 1.0 mg/kg of mitomycin C.
Metastases were not found. **Fig. 9** Lung **Fig. 10** Liver **Fig. 11** Kidney
Metastases were found. **Fig. 12** Lung **Fig. 13** Liver

Pathological findings in various organs in the group treated with chymotrypsin.
Metastases were found. **Fig. 14** Lung **Fig. 15** Liver **Fig. 16** Kidney

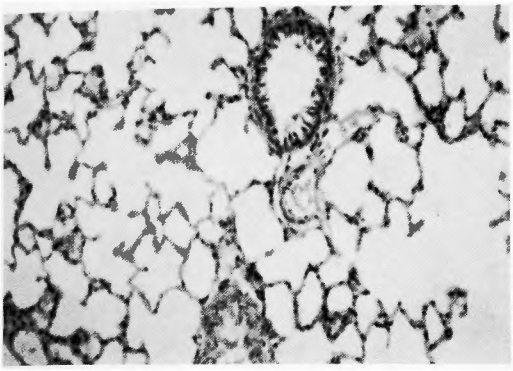

Fig. 1

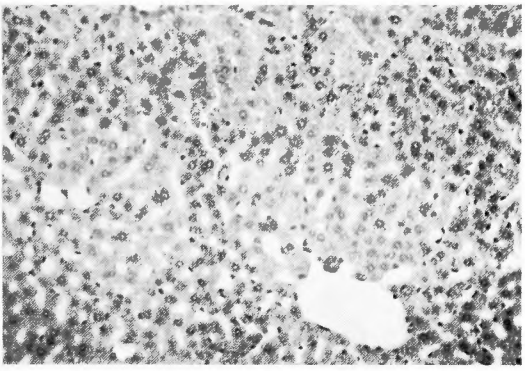

Fig. 2

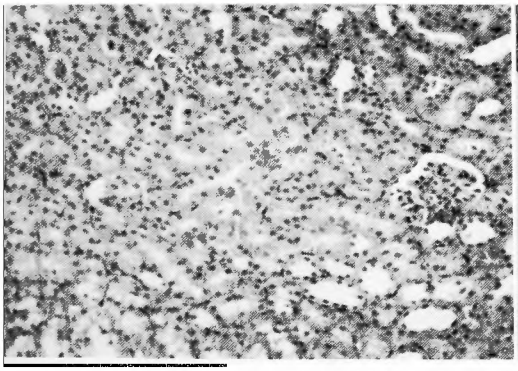

Fig. 3

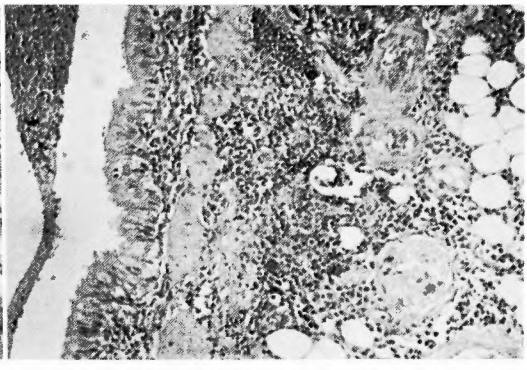

Fig. 4

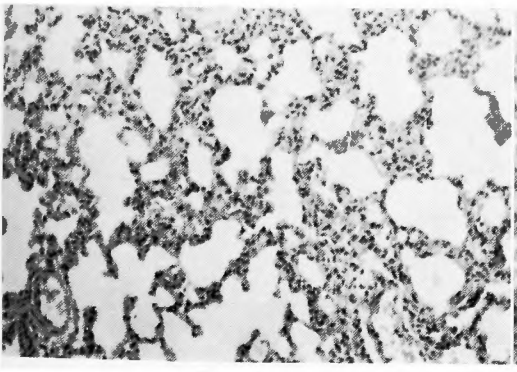

Fig. 5

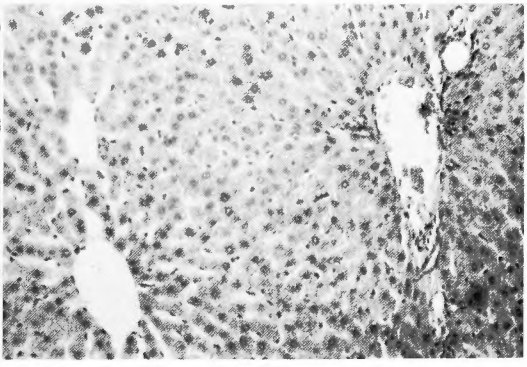

Fig. 6

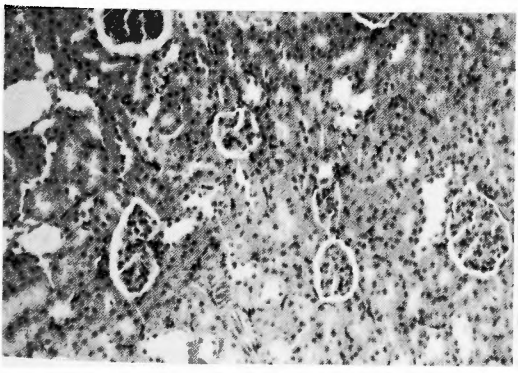

Fig. 7

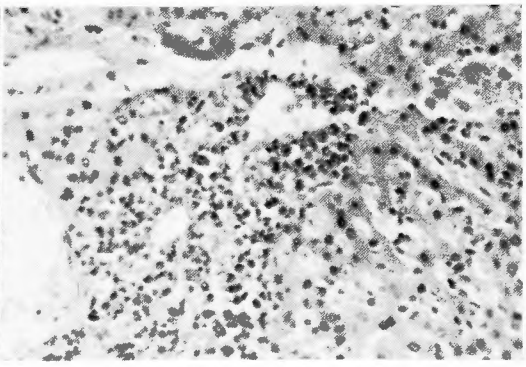

Fig. 8

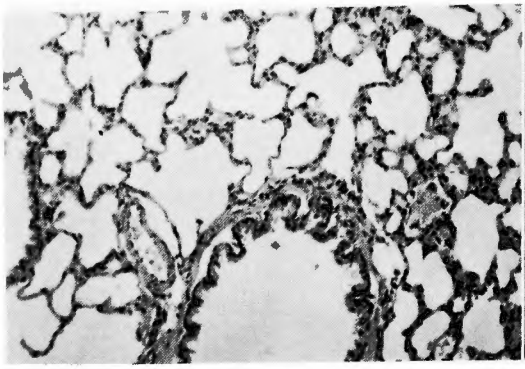

Fig. 9

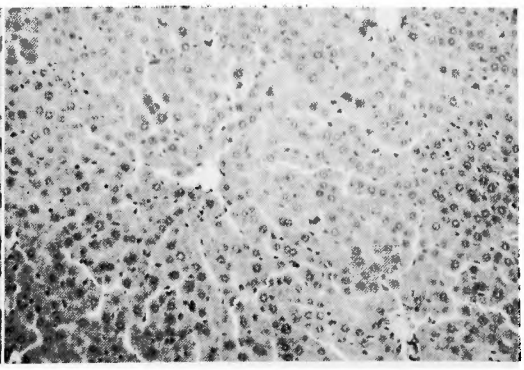

Fig. 10

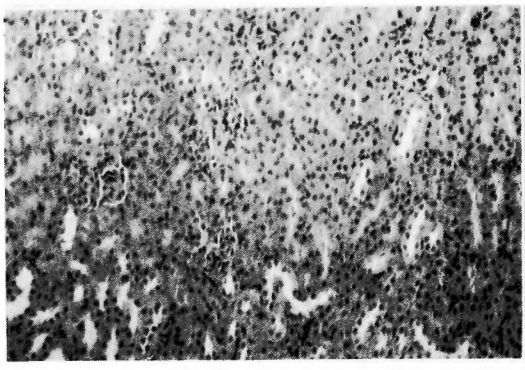

Fig. 11

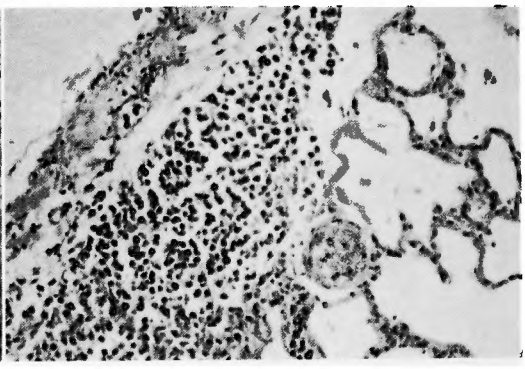

Fig. 12

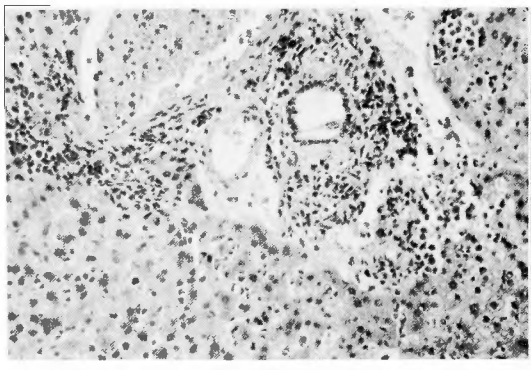

Fig. 13

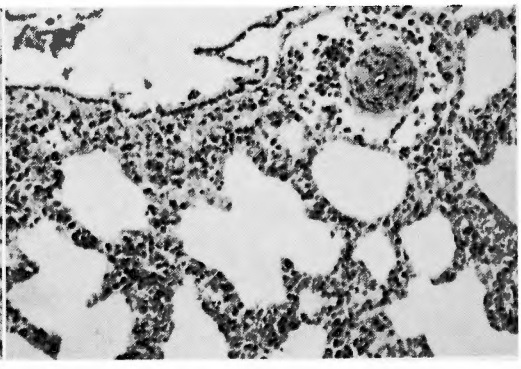

Fig. 14

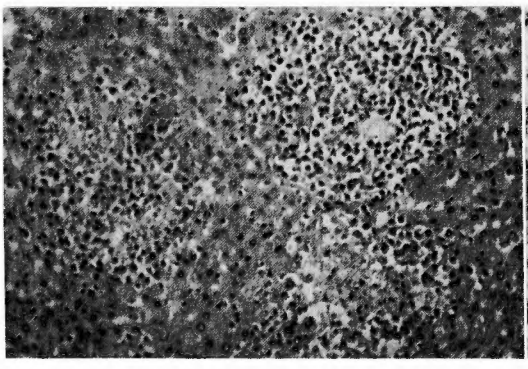

Fig. 15

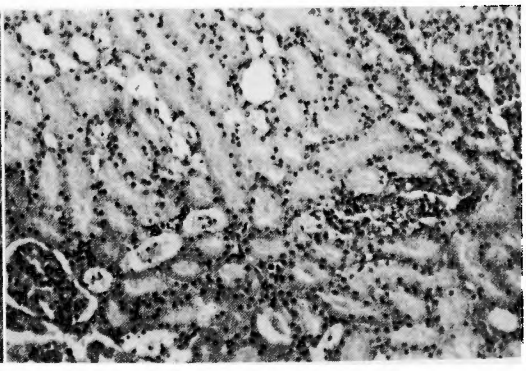

Fig. 16

和文抄録

血液凝固抑制剤と制癌剤の併用による悪性腫瘍の治療に関する実験的研究

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悪性腫瘍患者の流血中に腫瘍細胞が可成り高率に発見されるという事実は古くから多数の報告がある．しかしながら，これらの流血中の腫瘍細胞は必ずしも転移を起す素材になるとは限らず，一部は血管内で明らかに死滅することが知られている．流血中の腫瘍細胞が転移巣を作る場合の病理学的な過程を明らかにしたのはWoodである．彼によれば腫瘍細胞の着床には局所の毛細管におけるフィブリンの発生，小血栓の形成がみられるという．従つて転移の防止或いは比較的新鮮な小転移巣に対し化学療法剤を使用する際，血液凝固抑制剤を併用し，腫瘤形成に至らない間に化学療法剤を作用せしめることは悪性腫瘍の化学療法において興味ある問題を提示するものと思われる．

体重100g前後のドンリユウ系ラッテを用い股静脈より吉田肉腫細胞を注入し，同時に転移の防止或いは小腫瘍巣の治療を目的として血液凝固抑制剤と制癌剤の併用療法を行ない，生存率，剖検所見等を比較検討した．

1) Mitomycin C 単独使用群で，Mitomycin C の投与量を検討した結果1.0mg/kgの投与が最も生存率がよく，実験動物の8%において長期生存例が見られた．

2) Heparin 単独使用群では実験動物の10%において長期生存例が見られた．

3) Mitomycin C の最適量とHeparin を同時に併用すると実験動物の26.7%において長期生存が得られた．

4) 長期生存例については屠殺し諸臓器につき顕微鏡的に転移の有無を詳細に検討したが，HeparinとMitomycin C の併用群では約90%において腫瘍細胞を見出し得なかつた．尚Heparin 又は Mitomycin C の単独投与群の長期生存例では約60%において腫瘍細胞を見出し得なかつた．

5) Heparin投与群ではHeparin投与のためと考えられる出血傾向等は何等見出し得なかつた．

6) Chymotrypsinの使用は Heparin 投与時の様な成績が得られず，Heparin 投与の抗癌効果は腫瘍細胞の臓器着床の第一歩であると考えられるフィブリン生成の阻止に関係すると思われた．

本実験は血液凝固抑制剤と制癌剤の併用が悪性腫瘍の転移防止或いは小腫瘍巣の治療に極めて有力であることを実験的に示し得たものである．